ON AUTO-PARTHENOGENESIS IN ARBACIA AND ASTERIAS.

OTTO GLASER.

(From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.)

The unfertilized ova of *Arbacia* eliminate substances which discolor the sea-water, but when the same eggs are undergoing impregnation, the discoloration of an equal amount of sea-water in unit time, is roughly half again as much ('14[2]). Hand in hand with this fact goes the discovery that both *Arbacia* (*punctulata*) and *Asterias* (*forbesii*) ova, are measurably smaller in volume after fertilization than before ('13[1], '14[1]). These observations led me to begin an investigation which might throw some light, not only on the nature of the substances lost, but on the further question whether their elimination is significantly associated with the initiation of development. Not more than a beginning has been made, but some of the preliminary, chiefly qualitative, results are sufficiently interesting to be reported at the present time.

My earliest experiments consisted in a series of attempts to find what sort of substances could be gotten out of the eggs. I therefore prepared solutions in sea-water from ground, laked, or extracted ova. These preparations when tested with sperm exhibited the properties which had been described by F. R. Lillie for egg-secretion ('12, '13[2,3]). Emphasis must be laid on the power of these extracts to activate the sperm; on their chemotactic effect; and on their sperm-agglutinating as well as sperm-paralyzing capacities. Addition of the extracts in certain concentrations to normally fertilized eggs, resulted in a retardation of development; normal blastulæ instantly slowed their movements, and underwent a noticeable increase in volume when subjected to the extracts. Similar observations were made on the larvæ of *Arenicola* whose rate of movement was also slowed down, to be followed instantly by an outflow of their yellow pigment and a slight and reversible agglutination.

From these preliminary observations it appeared that the egg-extracts are capable of increasing the permeability of cells. On the view therefore that an increase in permeability is associated, significantly or otherwise, with fertilization, the initiation of development by means of these extracts should be possible. Against this idea stood the evidence of Loeb ('11) according to which "it was found that it was absolutely impossible to cause membrane formation of the sea-urchin egg by extracts from the sea-urchins of the same or even related species." From this Loeb concluded that the cortical layer of the egg exhibits against the lysins of the body producing it, the immunity characteristic for genetically related cells in general. It seemed little likely therefore that extracts prepared from the cells themselves should result in the initiation of development, and as a matter of fact, "membrane formation" in the sea-urchin egg did not occur. On the other hand, in corresponding experiments on the eggs of *Asterias*, a typical fertilization membrane did appear, and in both sea urchin and starfish, development was successfully initiated. For the type of initiation here dealt with, I have chosen the term auto-parthenogenesis.

Auto-Parthenogenesis in *Arbacia* by Means of Egg-Secretion.

The solutions employed in these early experiments, despite the use of weighed quantities of eggs, were not only very variable in concentration, but also differed in composition according to the method of preparation ('14[2]). Since the secretion from unfertilized eggs, has the same effects on sperm as the extracts, it appeared possible that it might also have similar effects on blastulæ, *Arenicola* larvæ, and unfertilized ova. This indeed was soon found to be the case, although as pointed out elsewhere ('14[2]) extract and secretion are probably not identical throughout.

The next step, the discovery of a more reliable mode of procedure, resulted in a definite method of securing results, but I have no reason for considering it final. Omitting the experiments of orientation and other unnecessary details, the following outline may be taken as a guide for further work: Standard secretion was prepared by adding to a certain number of "dry"

ripe ovarian eggs, double their volume of sea-water. At the end of ten minutes, during which the eggs were slightly agitated at intervals, the suspension was centrifugated, and the eggs cast down. After 100 revolutions the supernatant fluid was carefully decanted and set aside for use.

Ripe eggs were then shaken, usually from the ovaries of a single individual, into a small quantity of fresh sea-water, and to 1 c.c. of a concentrated suspension of these, was added 1 c.c. of the secretion. In this mixture the eggs were allowed to stand 2 hours, when cleavages were usually found in all the dishes. That these were not the result of accidental infection with sperm was guaranteed by special precautions—all dishes, instruments and the hands as well as the animals used having been carefully sterilized. Furthermore, when the first urchin opened was a female, I usually sought no further, as the eggs from a single individual are sufficient in number. Indeed in all but two or three series only eggs from a single urchin were used. It might be urged that the secretion or the sea-water were infected. Sterilization of the latter however does not prevent auto-parthenogenesis, and against the idea that the sperm might have been present in the secretion there are three arguments, two of which are conclusive. In the first place, as has been shown by F. R. Lillie ('13[2]) and myself ('14[2]), the fertilizing power of sperm decreases markedly if they are allowed to remain in the secretion more than a few hours. In some of my experiments the secretion was prepared a day in advance, and used on the eggs of the first urchin opened on the following morning. In the second place fertilization membranes never appeared in any of the experiments with *Arbacia* eggs unless especially induced, and lastly, if the same eggs from which the secretion was prepared were afterwards subjected to its influence for the proper length of time and in the proper concentration, characteristic auto-parthenogenesis took place.

Many experiments were tried varying the concentration of the secretion as well as the time of exposure. My records indicate cleavages at higher concentrations as well as lower, and also in less than two hours, but the greatest number was always obtained when 1 volume of the concentrated egg suspension was exposed

for 2 hours to 1 volume of the standard secretion. If at the end of this time the supernatant fluid is poured off and replaced by fresh sea-water, free-swimming blastulæ will be found within 24 hours. In one case only did development proceed to the pluteus stage.

Among the blastulæ that develop in these cultures are some characterized by an extremely slow rate of movement; others, though rare, may move at a practically normal rate, whereas the majority are considerably below normal in speed. This fact in earlier experiments misled me, as I caught sight of only the fastest swimmers, and mistook the others for arrested cleavages.

The size of the blastulæ is also a ready source of deception, since there are in general three kinds, micro-blastulæ, normal-sized blastulæ, and mega-blastulæ, of which the first are by far the most numerous. As these are usually very transparent and also slow of movement their presence in a culture may easily be overlooked.

The origin of the micro-blastulæ is clear. The first two cleavage cells, although normal in size, in most cases do not remain together, but separate and proceed to develop independently of one another. If the cells produced by these isolated blastomeres continue to separate very minute micro-blastulæ may result. Such falling apart of the cleavage products has been observed in the 16-cell stage and even later. The separation takes place, I believe, because the fertilization membrane has not appeared ('13[1]).

Normal-sized blastulæ need no explanation, since they must result from the eggs whose cleavage cells can be seen to hold together despite the absence of the normal fertilization membrane. As for the mega-blastulæ, it is possible that their origin is identical with that of mega-blastulæ in normal cultures. On the other hand, they were more noticeable in the auto-parthenogenetic series, and it is possible that the larger type of egg is better suited for this than the normal mode of development. Again the possibility is not excluded that the mega-blastulæ may come from eggs of normal size. This is not unlikely, since normal sized blastulæ instantly increase in volume when subjected to the same concentrations of the secretion.

Improved Method of Auto-Parthenogenesis.

Loeb's improved method of artificial parthenogenesis consists in following the treatment with parthenogenetic agents, by an after-treatment with hypertonic sea-water, 8 c.c. of 2.5 *M* NaCl + 50 c.c. sea-water. It seemed likely therefore that a better yield of larvæ could be secured if eggs, after having been subjected to the action of the secretion for two hours, were afterwards treated with the hypertonic solution for forty minutes. This surmise proved correct. The following is an outline of a typical experiment:

Time.	*A*	Time.	*B*
10.20 A.M.	1 c.c. eggs + 1 c.c. secretion.	10.12 A.M.	1 c.c. eggs + 1 c.c. secretion.
12.12 P.M.	Began hypertonic treatment.	1.12 P.M.	Began hypertonic treatment.
12.52	Ended hypertonic treatment.	1.52	Ended hypertonic treatment.
2.30	Considerable number of 2, 3, and 4-cell stages.	2.45	Many cleavages; 2–8 cells. Many normal.
5.45	Numerous 2, 3, and 4-cell stages.	6.00	Many 2, 4, 32, 64 and 128-cell stages.
	Very few 32–64-cell stages.	7.30	Cleavages in every field.
9.30 A.M.	1 active larva, many dead.		Some blastulæ but not free.
		9.45 A.M.	12 fine active larvæ. 50 per cent. of cleavages arrested.
		4.00 P.M.	Hundreds of slowly moving blastulæ.

In experiment *B* the time of exposure to the secretion was three hours instead of two as in earlier experiments with the hypertonic after-treatment. From this one might infer that better results could have been obtained in the experiments in which the secretion alone was used, but the results do not warrant this assumption. Controls also show that with the hypertonic after-treatment, three hours of exposure to the secretion is better than two. We must believe therefore that the success of experiment *B* and all the others carried out in the same way, depended on the hypertonic after-treatment.

The differences in the length of exposure to the secretion are for the present purely empirical.

In all the experiments both with as well as without the hypertonic after-treatment, development was much delayed in many

of the eggs, and rarely went beyond the blastula stage. As stated before, I observed plutei only once, and not over ten or twelve at that. Gastrulæ also were rare. For this failure to pass beyond the blastula stage there are several reasons; in the first place one must attribute a large share to the fragmentation of the early cleavage stages, due as I believe to the absence of a normal fertilization membrane, and secondly the abnormal permeability relations resulting from the treatment with the secretion probably also play a rôle ('14[2]) by placing mechanical difficulties in the way of gastrulation even though the necessary materials are present in the embryo. The evidence for this conclusion cannot be presented now. Although these obstacles stand in the way of perfecting this method of rearing sea-urchins, the fact that it is impractical, and inferior to other methods does not in the least detract from the theoretical interest of the results. As McClendon ('09) puts it: "In natural parthenogenetic development the end result may be a maturation or segmentation stage or a larva or adult. Though only the reproductive adults are of significance to the species, all are of significance to science."

Auto-Parthenogenesis in *Asterias*.

In earlier papers ('13[1], '14[1] and '14[2]) I have emphasized certain differences as well as certain points of similarity between the eggs of *Arbacia* and *Asterias*. Chief among the differences, are the characteristics of the fertilization membranes; most important among the points of likeness are the decrease in volume to be observed in both kinds of eggs on fertilization, and the fact that the *Asterias* ovum produces a secretion in which the most striking characteristics of the corresponding *Arbacia* exudate can be verified point for point. For these reasons one might anticipate the initiation of development in the *Asterias* egg by the proper employment of its own secretion.

In the following experiments the secretion was prepared exactly as in the case of *Arbacia*, but the eggs before treatment were given time to mature, *i. e.*, they were allowed to stand for about 1 hour in sea-water after removal from the ovaries.

10.27 A.M.	1 volume *Asterias* eggs. 1 volume *Asterias* secretion.	10.27 A.M.	2 volumes *Asterias* eggs. 1 volume *Asterias* secretion.

10.50	Fertilization membranes indicated.	10.53	Fertilization membranes indicated.
12.15 P.M.	Many eggs elongated.		
2.00	Fertilization membranes. Frequent cleavages.	2.00 P.M.	Frequent cleavages.
9.00 A.M.	Cytolysis. One free swimming blastula.	9.00 A.M.	Numerous dead blastulæ. Much gastrulation.

In other tests similar results were obtained. Unfortunately this work came at the end of the *Asterias* breeding season, and further experiments were impossible. Those performed however are sufficient to show that parthenogenesis can be induced in *Asterias* as in *Arbacia* by the use of its own egg-secretion. Owing to the existence of a perivitelline space within which the cleavage products of the *Asterias* egg are held, it appears likely that better results will ultimately be achieved than in the auto-parthenogenesis of *Arbacia*. In fact as indicated above, even without hypertonic after-treatment, which in first experiments was always omitted for the sake of control, one of my auto-parthenogenetic *Asterias* cultures proceeded to the gastrula stage.

"Hetero-Parthenogenesis."

"Hetero-parthenogenesis" as the sequel will show, is probably not the correct word, but I use it here as a convenient heading for experiments in which development was initiated in *Asterias* eggs by the use of *Arbacia* secretion. Such experiments suggested themselves, as the result of the earlier observations on the increase in the permeability of various types of cells. From these one might anticipate "hetero-parthenogenesis," a process which was indeed successfully induced as the following experiment indicates:

10.35 A.M.	1 volume mature *Asterias* eggs.
	1 volume *Arbacia* secretion.
	No immediately visible effect.
10.50	Fertilization membranes indicated.
2.00 P.M.	Numerous cleavages.

The Fertilizin Theory.

After I had discovered auto-parthenogenesis through the observation that the egg-extracts are permeability increasing agents, I consulted with Professor F. R. Lillie who had been performing

those experiments on normal fertilization in *Arbacia* which have led him to propose the fertilizin theory, briefly outlined in his paper "The Mechanism of Fertilization" ('13[3]).

The facts on which this theory is based are dealt with in F. R. Lillie's earlier papers, as well as in the one specifically referred to. In my paper on "A Qualitative Analysis of the Egg-Secretions and Extracts of *Arbacia* and *Asterias*" ('14[2]) will be found certain verifications of Lillie's observations. It would take us too far to go into the factual basis of the theory here, but briefly it postulates as the essential point in fertilization a chain of chemical reactions in which an amboceptor-like substance—the sperm-agglutinating agent—present in the secretions from the egg, unites by means of a spermophile side-chain with receptors in the sperm, and by an ovophile side-chain with receptors in the egg.

If this chain of reactions indeed occurs and is related to fertilization and the initiation of development in the significant manner put forward by Lillie, it follows that there are theoretically at least five possibilities of blocking fertilization. Lillie has listed these as follows:

1. Through loss of fertilizin by the egg.
2. Through occupancy of the sperm receptors.
3. Through occupancy of the egg receptors.
4. Through occupancy of the ovophile side chain of the amboceptor (fertilizin).
5. Through occupancy of the spermophile side-chain of the amboceptor (fertilizin).

Of these Lillie has proved possibility 1, by washing the eggs; 4, by means of an inhibitor in the blood which prevented fertilization, but not the sperm-agglutinin reaction; and 5, by setting free from the egg itself an inhibitor, the anti-fertilizin which obstructs the union of the sperm with the amboceptor by occupying the spermophile side-chain. Since therefore the presence of the agglutinating agent, and moreover its presence in a state in which both spermophile and ovophile side-chains are free to combine with their respective normal receptors in the sperm and the egg, seems to be necessary for normal fertilization, Lillie has called the substance in question, fertilizin.

F. R. Lillie has also offered an interpretation of my results on auto-parthenogenesis. He suggests that in the initiation of development by employment of the egg-secretion, essentially the same chemical chain is involved as in normal fertilization except that the sperm, and naturally the sperm-fertilizin reaction also, is dropped. In other words, in auto-parthenogenesis, the egg-receptors are thought of as combining with the ovophile side-chains of fertilizin which has not been bound to sperm-receptors through its spermophile groups.

Off-hand this supposition does not appear unreasonable. The fertilizin is certainly present in abnormally high concentration in the secretion as used for auto-parthenogenetic initiation, and the mere fact that the secretion is a permeability-increasing agent does not preclude the possibility that one of its constituents may play an additional rôle in the initiation of development. If this is true, it should be possible to block auto-parthenogenesis in accordance with the scheme outlined above, although the absence of the sperm and the sperm-fertilizin reaction restricts the opportunities to 1, the loss of fertilizin; 3, the occupancy of the egg-receptors; and 4, the occupancy of the ovophile side-chain of the fertilizin.

Methods of Preventing Auto-Parthenogenesis.

1. *The Prevention of Auto-parthenogenesis by Washing the Eggs.* —Several preliminary experiments in which the eggs before being exposed to the action of the secretion were washed from 20 minutes to half an hour in great excesses of sea-water, proved conclusively that this procedure very materially reduces the number of cleavages, and the number of larvæ, afterwards found in the cultures. These results led to a more careful test carried out on eggs which had been washed in excesses of sea-water for 24 hours. The sea-water was changed frequently but at irregular intervals. These eggs were then treated in the usual way with the secretion, but not a single cleavage was found. Thus the effect of even slight washing is noticeable whereas prolonged washing effectually prevents the auto-parthenogenetic cleavage. From this we might conclude that something essential for the initiation of development had been removed from the eggs.

However the failure of the fertilizin with which the eggs were treated and for which they are permeable, to offset the effects of washing, remains to be accounted for.

2. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Sperm.*—Lillie has made the apparently justifiable assumption that fertilizin bound by sperm is activated, *i. e.*, has a greater chemical affinity for the substances with which it unites in the egg than the unbound fertilizin. If this is true, it occurred to me that the use of sperm, in conjunction with the secretion might give better auto-parthenogenetic results than had been obtained by the use of the secretion alone, either with or without the hypertonic after-treatment. Naturally only dead or seriously injured sperm are available for this experiment, and with the latter one could not be sure to exclude a certain and misleading number of real fertilizations which would then be mistaken for very fine cases of parthenogenesis. Since the power of the fertilizin to produce the agglutination reaction and to play a part in normal fertilization is not destroyed by brief boiling, it seemed possible that the complementary chemical groups in the sperm might also survive boiling without being destroyed or so altered as to be incapable of taking some part at least in the reactions between egg and fertilizin.

However, instead of furthering auto-parthenogenesis, exactly the opposite effect was found, for the boiled sperm absolutely prevented the process, as shown by the following experiment:

1 volume of eggs+1 volume of secretion[1]+2 volumes boiled sperm infusion 10.40 A.M. to 2.50 P.M. Four hours later little if any effect visible; a few cell-masses were noted, but nothing that in any way suggested auto-parthenogenesis. At 2.58 P.M. the mixture was diluted with an excess of sea-water. At 6 P.M. no effect was noticeable; by 10 A.M. the next day, the eggs had undergone a granular decomposition.

Experiment repeated carefully controlled. Result identical. In the control however auto-parthenogenesis occurred and on the following morning 12 very fine larvæ were seen in addition to the usual number of delayed cleavages and micro-blastulæ.

[1] Unfortunately the egg-secretion in these tests was more dilute than usual, but the effect noted is not interpretable on this basis since parthenogenesis occurs at greater dilutions. The sequel to these experiments further emphasizes the interpretation given here.

Since the fertilizin theory postulates essentially the same machinery for auto-parthenogenesis and normal fertilization it follows that initiation by living sperm should also be blocked by treating the eggs with the boiled sperm-infusion. This is actually the case:

Eggs treated for two hours with strong boiled sperm-infusion. At the end of this period they were heavily inseminated with living sperm, but remained unfertilized. Only a few cleavages were observed later, but these were arrested at the latest in the four-cell stage. No further development occurred, but on the following day practically all the eggs exhibited fertilization membranes. Controls normal.

3. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Secretion.*—F. R. Lillie ('13[2]) finds the agglutinative power of egg-secretion unimpaired, or perhaps better, not measurably impaired, by boiling for 5 minutes, although the solution undergoes a change of color due to the formation of small quantities of a purple compound ('14[2]). As the secretion is thus visibly different in chemical composition it becomes important to ascertain whether the formation or presence of the purple compound makes any recognizable difference in the initiation of development. The following experiments indicate that the efficacy of the secretion as a parthenogenetic agent is blocked.

Standard secretion boiled. Purple compound formed. Sperm-agglutination positive.

3.20 P.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

8.00 P.M. No cleavages.

8.00 A.M. Few delayed cleavages, not at all in normal frequency.

11.30 A.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

2.00 P. M. No cleavages.

7.00 P.M. No cleavages.

8.30 A.M. No cleavages.

Control in unboiled secretion exhibited auto-parthenogenesis in usual quantity.

Interpretation of Experiments on Prevention of Auto-Parthenogenesis.

Analysis of the means by which a process can be prevented may be even more suggestive than a similar analysis of the means by which it can be induced, and this proves to be true in the present case. How then are these experiments to be understood?

1. *The Block in Washed Eggs.*—The prevention of fertilization by washing (F. R. Lillie, '13[3]), according to the fertilizin theory, is due to the absence of the intermediary body with which both sperm and egg receptors normally unite. We cannot, however, despite the superficial similarity of the two cases, attribute the auto-parthenogenetic block to the absence of suitable bonds for the egg receptors since the surface film of the ovum is perfectly permeable for fertilizin. This must diffuse into the eggs from which it has been removed the moment they are exposed to the concentrations employed for initiation. If this is correct, then the prevention of auto-parthenogenesis in washed eggs must be explained in some other way. This however is not necessarily fatal to the fertilizin theory, for these same experiments strongly suggest that a mere increase in permeability is insufficient to initiate development, a suggestion still further reinforced by the second type of block to be considered presently.

The experiments with washed eggs are also suggestive from another standpoint. Lillie (*loc. cit.*) tells us: "If it be true that the egg contains its own fertilizing substance it might also be possible to induce parthenogenesis by increasing the concentration of this substance to a certain point. . . ." The washed eggs however demonstrate that the fertilizin experimentally added to the solution about the eggs has no fertilizing effect, and if it has none when the eggs have been washed, there is no good ground for considering its effect different when the eggs have not been washed. "Hetero-parthenogenesis" strongly backs up this position. It appears most likely therefore that in auto-parthenogenesis the fertilizin experimentally added, plays the same rôle as any other permeability increasing agent effective in the artificial initiation of development. If this is correct, and furthermore, if the parthenogenetic block in washed eggs is not identical with the fertilization block brought about by the same means,

it follows, not that the fertilizin theory is inapplicable to these cases, but only that it does not apply exactly as F. R. Lillie has supposed.

2. *The Block by Means of Groups Split from the Sperm and the Egg-Secretion.*—The prevention of both fertilization and auto-parthenogenesis in eggs previously treated with boiled sperm infusion, as well as the parthenogenetic block in eggs subjected to the action of boiled secretion, seem at first sight to have little in common. In reality however there is an element of identity in all three cases, for suspensions of sperm as well as the egg-secretion give on boiling a characteristic color reaction due to the formation of a purple substance which may be looked upon as a chemical cleavage product split off from a mother substance. In an earlier paper ('14[2]) I suggested on other grounds, and in complete ignorance of its rôle in parthenogenesis, that this purple material might prove to be significant, as it seems to be specific for *Arbacia*. This suggestion now appears almost certainly correct, and I therefore propose for this substance, provisionally at least, the name "Purple X."

An attempt to understand the part played by Purple X in these reactions must begin with the proof that this substance and not some other cleavage product is really the effective one. Such proof would be found if the block to both parthenogenesis and fertilization could be removed with the removal of the Purple X. To accomplish this and at the same time not render the solutions toxic by the addition of precipitating agents, called for methods which had first to be discovered.

The Purple X is either in a state of very fine suspension, bordering on solution, or is actually in solution. Since now a precipitable colloid frequently carries other bodies with it when thrown down, I added the albumen of hen's egg to the *Arbacia* egg-secretion, and boiled the mixture. The expectation that the purple compound might be removed at least to a considerable extent, proved correct, as the white coagulum of hen's egg albumen was quite markedly discolored by the purple substance. After filtration the secretion was tested for its parthenogenetic effect. The method of separation however is not quantitative and so the results were not perfectly clear cut. Nevertheless it

remains true that the only boiled secretion that ever induced parthenogenesis was one from which the Purple X had been removed by coagulating white of hen's egg. This result, I may add parenthetically was obtained only after a very remarkable effect of the egg-white itself had been investigated. Even in the dilutions in which it is present in the sea-water after boiling, the white of a hen's egg produces very regular and curious distortions of the eggs which may even be divided into pseudo-cleavage products. However the effect does not seem to be analogous to parthenogenesis, and I shall therefore deal with it separately at another time.

In the course of these experiments a very much better and simpler method was found, for the Purple X is a relatively unstable compound and readily disappears. Thus a concentrated sperm-suspension boiled on one day and giving a color as deep as port wine, may on the next be golden yellow. With such a solution eggs were treated for one hour and five minutes. At the end of that time the addition of living sperm resulted in the fertilization of practically every egg. The experiment was repeated, the eggs being exposed for two hours and forty minutes, and again fertilization occurred.

As a counterpart to these experiments with sperm, the parthenogenetic effect of boiled secretion which had lost its purple color was tried. Such solutions contrast sharply with the newly boiled secretion, for whereas the latter very effectually blocks auto-parthenogenesis, development can be induced without difficulty after the spontaneous disappearance of the Purple X.

Since the Purple X-block occurs in the presence as well as in the absence of sperm, we can be certain that it is not the result of an effect upon the male sex cells or, in the language of the fertilizin theory, of occupancy of the sperm receptors. Which particular one of the remaining three blocks theoretically possible we are dealing with however must for the present remain an open question, although if the immediate effect of the egg-secretion is really identical with the immediate effect of any other parthenogenetic agent, occupancy of the egg-receptors is indicated. If this is true, then Purple X should block parthenogenesis no matter how induced. That this process should be blocked by

less than is required to prevent fertilization is perfectly intelligible on Lillie's assumption that sperm-bound fertilizin is activated and has a greater affinity for the egg-receptors than free fertilizin.[1]

An Attempt to Construct a Provisional Working Hypothesis.

Although no final interpretation of the phenomena of fertilization is possible at the present time, an attempt to formulate some viewpoint which does no violence to our knowledge, and which at the same time may serve as a working hypothesis, must be made for further guidance.

If we accept the evidence of F. R. Lillie, together with my verifications and additions to it, and remember that many of the facts discovered by Lillie, as well as some found by myself, are the direct products of the fertilizin theory considered as a tool, I think we may even now admit that it is a useful working hypothesis. Lillie ('13) himself has emphasized this point, and moreover has listed among the other advantages of his theory, that it gives us an explanation of the specificities of fertilization; may furnish the foundations for the chemical conceptions needed by any theory of fertilization, and above all, that it offers one explanation for the initiation of development, whether by fertilization or parthenogenesis.

To what extent these claims are justified, and to what extent the theory will undergo modification, it would be unprofitable to discuss at present. One claim and its consequences however does invite discussion at the present time, and in this connection, for if the fertilizin theory is really capable of unifying the various methods of inducing parthenogenesis, its relations to another view, which apparently does the same thing must be analyzed, and furthermore we must answer the question whether all cases of parthenogenesis are really instances of auto-parthenogenesis.

[1] Since Purple X also appears when spermatozoa come into contact with the secretion, it is possible that its appearance plays a rôle in the prevention of polyspermy. This would not prevent a similar effect on the part of the anti-fertilizin, as suggested by F. R. Lillie. Indeed the chances that any particular sperm will complete the reactions postulated by the theory, must be exceedingly small when we reflect on the immense numbers which collect about each egg. Such collections become more intelligible when we recall that whereas the biparental effect can be carried out by a single sperm, the initiatory effect, at least in *Arbacia* ('13[1]) requires more than five.

1. *The Surface-Alteration Theory.*—The view which we may call the surface-alteration theory is a product of the work of Loeb, R. S. Lillie, and many others. According to Loeb the initiation of development depends in the first place upon a superficial cytolysis, or destruction of the cortical layer of the egg, brought about either by a lysin in the sperm, or by the so-called parthenogenetic methods and agents. After this alteration of the surface the rate of oxidation in the egg is raised from four to six times. Loeb says:

"There are two possibilities by which this result can be produced: either a catalyzer (an oxidase) is carried into the egg by the spermatozoon; or the change in the surface layer itself causes the increased rate of oxidation. Everything speaks in favor of the second assumption."[1] Further on Loeb suggests "that the spermatozoon causes development . . . by removing an obstacle to development."[2]

R. S. Lillie ('11 and earlier papers) has suggested that the superficial cytolysis increases the permeability of the egg, and further that through this increase in permeability a substance antagonistic to oxidation is eliminated from the egg. This substance he imagined might be CO_2 ('09).

Against this idea Loeb has raised objections. Inasmuch as CO_2 "is a good agency for calling forth membrane formation" and as only substances capable of diffusing into the egg can have this effect, the egg surface must be permeable to CO_2 before fertilization, and consequently there can be no accumulation that could be relieved as the result of an increase in permeability.

This criticism seems valid, and while it constitutes a reason for giving up the idea that the antagonist lost is CO_2, it in no way bears on the other idea, namely that there is an increase in the permeability of the ovum as the result of fertilization. However this idea also has failed of acceptance by Loeb, but this is due

[1] "Artificial Parthenogenesis and Fertilization," 1913, p. 13.

[2] The obstacle "removed" is a cortical layer unsuited for development, and the "removal" is in reality an alteration of this cortex: "Through cytolysis of the cortical layer of the egg the oxidations in the unfertilized egg are accelerated from four to six times their normal rate" (loc. cit., p. 14). Since this heightened oxidation persists after fertilization, and since the permeability of fertilized eggs may not be different from that of unfertilized, it is possible that the cytolysis of the cortical layer alone is insufficient to account for the increase in the rate of oxidation.

chiefly I believe because the evidence in support of it has been unfortunate not only in a purely technical sense which has been justly criticized, but also because it was gathered under a misapprehension apparently as to exactly what R. S. Lillie had suggested. So far as I can see, experiments in which the permeability of fertilized eggs is compared with that of unfertilized have no bearing on the question, for no such difference was postulated, but only that the "increased permeability is . . . temporary in normal or in favorable parthenogenetic fertilization" ('11, p. 307).

If this idea is correct we might expect two things to happen at fertilization in the egg of *Arbacia*. Since this egg before impregnation actively secretes materials that discolor the sea-water, the discoloration produced by eggs undergoing fertilization might be greater. I have found ('14[2]) that it is one and a half times that of eggs not undergoing impregnation. Further, fertilized eggs, after the process is complete, do not discolor the sea-water. In the second place even a temporary increase in permeability should result in a change in volume if the eggs remain in a medium that remains constant during the period under discussion. Measurements show ('14[1]) that the eggs of both *Arbacia* and *Asterias* are smaller in volume after fertilization than before.

I therefore consider the idea of increased permeability *during* fertilization correct, and that we may suppose all cases of fertilization, whether with sperm or without to have this one point in common. Since many of the results now published had not been found at the time I wrote my preliminary paper ('13[1]) I threw emphasis on this common attribute in section VIII. This emphasis I do not wish to withdraw as the result of later work, for this has only strengthened my conviction that in fertilization an increase in the permeability of the ovum actually occurs. The only question at issue seems to me to be the significance of this change for the initiation of development.

On this particular point I also postulated the loss of substances antagonistic to oxidation. This suggestion was based on the fact that substances are lost during the act of fertilization at a higher rate than before, and finally that the presence of egg-secretion in certain concentrations retards development. The

retardation I supposed to be due to the depression in the rate of oxidation. This assumption, as I pointed out, does not seem unreasonable; on the other hand as I pointed out more recently, retardation of development may in this case also be due to interference with, or depression of, other processes no less essential. Only further work can decide these questions.

2. *The Fertilizin Theory.*—In the fertilizin theory, no particular rôle is assigned to the increased permeability. "The nature of the effect of the activated fertilizin on the egg is analogous in some respects to a superficial cytolysis, in this respect agreeing with Loeb's theory. But the 'lysin'[1] is contained in the egg, not in the sperm as Loeb thought; if cytolysis is involved, it is a case of auto-cytolysis. This may involve increase of permeability, the effects of which R. S. Lillie especially studied" ('13[3]). These possibilities were mentioned "in order to point out that the conception" of the fertilizin theory "is not in conflict with the well-established work of others." This is very important for the fertilizin hypothesis is based on certain facts which seem to permit the interpretation given them, and the surface-alteration theory also has a verifiable factual basis. These facts, occurrences in and about fertilization, cannot be in conflict with one another. The problem is to understand their interrelations. The experiments on auto-parthenogenesis carry us a step forward in this direction.

At first sight success in initiating the development of *Asterias* eggs by means of *Arbacia* egg-secretion would appear to seal the death-warrant of Lillie's fertilizin theory. On the other hand a little reflection will show that no radical conclusions need be drawn. Parthenogenesis, as is well known can be induced in different eggs by the most diverse means—electricity, heat, cold, mechanical shock, osmotic pressure,[2] specific chemical alteration

[1] It would have been more correct to say: it *was* contained in the egg.

[2] In the case of the purely osmotic methods, Loeb supposes that "the hypertonic solution acts simultaneously in two capacities: first as a cytolytic agent causing a change in the cortical layer (the formation of a gelatinous film) and second, as a corrective agency" ('13[4], p. 159).

The cytolytic action of the hypertonic solution is not without evidence, and so we may consider this method as having the effect common to all the others. If however the increase in permeability is effective also on account of the destruction of the normal fertilization block, in the sense outlined in the text, loss of water by the egg would directly or indirectly accelerate the loss of the antagonists (cp. Glaser, '13, p. 450).

of the surface film or cortical layer, lipoid solvents, and even the prick of a needle having been found effective. These methods, each of which is capable of variation and modification, have nothing in common except their result, and it is idle to try to imagine any direct causal connection between them and the result. However one effect, common to all, seems to stand intermediate between the parthenogenetic result, and the experimental means used to induce it. This common intermediary is a change in the permeability of the surface of the eggs, and in the experiment in which parthenogenesis is induced in one ovum by the secretion of another it is not necessary to assume that anything occurs that does not also take place in all the other cases. The same reasoning applies also to auto-parthenogenesis, a process which was found by application of the surface-alteration theory, and in total ignorance of the fertilizin hypothesis.

Now the picture of fertilization with sperm given by the fertilizin theory is briefly the sperm-receptor-amboceptor-egg-receptor reaction. In the picture of auto-parthenogenesis, the sperm is dropped, leaving the amboceptor-egg-receptor to react, the former with an unsatisfied bond. Since however parthenogenesis can be induced without the addition of fertilizin to the eggs there is no reason for supposing that the egg-receptors united with the fertilizin which was added in my experiments for the purposes of initiation. This however does not seem to me to be at all necessary, for both substances are present in the egg from the beginning and we may postulate a union between the receptors and the fertilizin within the egg.

If this is indeed the mechanism of both parthenogenesis and auto-parthenogenesis, we must answer the question why the egg was not fertilized before, or what amounts to the same thing, why fertilization occurs at all.

In speaking of parthenogenetic agents, Lillie (*loc. cit.*) tells us, they "need only remove obstacles to the union of the amboceptor and the egg-receptor" (p. 527); and in connection with auto-parthenogenesis, that this might result "if the concentration of fertilizin were raised to a certain point, though it is conceivable that no increase in concentration would break down the resistance that normally exists to the union of the amboceptors and egg-

receptors" (527). In other words, the egg is not always fertilized, because there are obstacles or resistances. When these are removed, fertilization, parthenogenesis, or auto-parthenogenesis may occur.

No circumstance connected with the initiation of development that is not a common occurrence in all cases is capable of explaining how these obstacles or resistances can be removed. It is not necessary for clear thinking on this subject to know what they are at present, but the only common fact which seems capable of explaining how these natural blocks to fertilization, and the initiation of development could be removed, is at present the increase in the permeability of the ovum. For this reason, without in any way trying to minimize the significance of the facts emphasized by the fertilizin theory, I believe that the permeability change plays a very real part in the process of fertilization, for through it is possible a disturbance of the equilibrium in which we know the egg finds itself prior to impregnation or parthenogenetic initiation.

If this viewpoint is correct, the following picture of both fertilization and parthenogenesis is possible. Fertilizin alone in sufficient concentrations is capable of increasing the permeability of the ovum; smaller quantities when activated by union with sperm are able to exert the same effect. This alteration of the surface may also be brought about by electricity, heat, cold, mechanical shock, specific chemical alteration of the membrane, lipoid solvents, pricking, or any other way of rendering the egg surface more permeable or of accomplishing the same result as an increase in permeability. The immediate effect of this permeability increase is a removal of those obstacles or resistances which constitute the normal fertilization block. These removed, the fertilizin within the egg combines with the egg-receptors, but if fertilizin activated by sperm is present, the egg receptors would combine with the elements for which ex hypothese they have the greater affinity.

So far the facts seem to group themselves harmoniously, but how are we to conceive of the "obstacles" or "resistances"? We might imagine the existence within the unfertilized egg of a body capable of playing the required rôle, but for this assumption

there is no evidence. Moreover the substances demonstrated by F. R. Lillie, seem to me sufficient to account for the normal fertilization block if we make a much simpler assumption—one indeed which appears necessary on chemical grounds.

The ovum, according to Lillie, contains egg-receptors, fertilizin, and antifertilizin. Chemical affinities exist between the anti-fertilizin and the spermophile groups as well as between the receptors and the ovophile groups. Although Lillie does not postulate a reaction involving these elements in the unfertilized egg, it is difficult to believe that three substances, demonstrably capable of uniting in the manner indicated, would lie side by side without entering into combination, however loose. If this assumption prove valid, we can consider this union as constituting the normal fertilization block ("resistance," "obstacle") of the unfertilized egg.

From this standpoint it appears possible to explain the block to parthenogenesis in washed eggs. The egg surface prior to fertilization or treatment with parthenogenetic agents, is perfectly permeable to fertilizin. Lillie has shown, however, that the anti-fertilizin can only be removed by destruction of the ova, whose surfaces, with respect to these two substances, appear to act like dialysers. It is quite likely therefore that washed eggs differ from unwashed by a relatively higher concentration of anti-fertilizin. If now fertilizin is permitted to diffuse into a washed egg, it would be bound by the anti-fertilizin and so an initiatory effect, due to its further union (if indeed this occurs[1]) with the egg-receptors would be prevented. According to Lillie's view and the law of mass action, auto-parthenogenesis might occur in washed eggs when the concentration of fertilizin is more than enough to bind the anti-fertilizin. That it takes about two hours to initiate auto-parthenogenesis, and that I failed to initiate it in that length of time in washed ova, are both in harmony with the above suggestion.

So far as I can see, this is the only way at present, in which it seems possible to draw a picture in which all the facts and all the cases may find a place. It follows from the preceding reason-

[1] The avidity of fertilizin bound by sperm for the egg-receptors is assumed by Lillie to be greater than in unbound fertilizin. The opposite effect, resulting from a union of the spermophile groups with the anti-fertilizin is not excluded.

ing, as well as more directly from the experiments themselves, that the rôle of the egg secretion (fertilizin) experimentally added to induce development was exactly the same as the rôle of any other parthenogenetic agent, and that it united with the egg receptors, no more than do the electricity, heat, cold, mechanical shock, osmotic pressure, or what-not. On the other hand, like these, the egg-secretion by affecting permeability, brings about conditions under which the ovum is freed from its inhibitors and, in a sense, enabled to fertilize itself. From this standpoint all parthenogenetic methods are methods for inducing auto-parthenogenesis, and fertilization with sperm differs from auto-initiation simply in the fact that the fertilizin which unites with the egg receptors has itself previously united with the sperm in the jelly, or the water about the egg.[1]

Summary.

1. The egg-extracts as well as the egg-secretions of *Arbacia* and *Asterias*, in addition to characteristic effects on spermatozoa, are permeability increasing agents, and initiate development. For the type of initiation here dealt with I have chosen the term *auto-parthenogenesis*.

2. Auto-parthenogenesis is blocked in *Arbacia* by washing the eggs in sea-water.

3. A group "Purple X" which may be split off from the sperm of *Arbacia*, and which also appears in boiled egg-secretion, blocks parthenogenesis in low concentrations and fertilization in high concentrations.

4. Prevention of parthenogenesis by washing can hardly be the result of an absence of fertilizin; prevention by Purple X may be the result of occupancy of the egg-receptors.

5. It is not unlikely that all cases of parthenogenesis are in reality instances of auto-parthenogenesis.

6. With certain minor modifications it appears possible to

[1] According to F. R. Lillie's account, the sperm unites with the fertilizin in the egg. This however seems incredible, for the affinity between sperm and fertilizin is very great, and since the egg jelly, through which the sperm must pass on their way to the ovum is heavily charged with the fertilizin, it is difficult to see how any unbound sperm could reach the egg. This change in the geographical location of the sperm-fertilizin reaction however makes absolutely no difference in essentials.

construct an hypothesis of fertilization in which the basal facts of the surface-alteration theory, as well as the facts emphasized by the fertilizin theory may, without violence to our knowledge, find a place. From the standpoint here adopted, there is no antagonism between the two theories which emphasize different groups of facts associated with the process of fertilization. These facts cannot be in conflict with one another.

UNIVERSITY OF MICHIGAN,
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